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EDITORIAL REVIEW

Mercury: god of Th2 cells?

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A complex mixture of affiliations is attributed to the Roman god Mercury: he was a messenger, the god of science and commerce, the patron of travellers but also of rogues, vagabonds and thieves [1]. It could be argued that in experimental biology inorganic mercury acts as a messenger with a similarly mixed set of respondents: in higher doses, mercuric chloride ($HgCl_2$) is toxic, leading to the death of metabolically active cells such as renal tubular cells [2]. In lower doses, $HgCl_2$ has potent effects on the immune system of rodents, the outcome being immune stimulation or immunosuppression depending on the strain of animal involved [3]. In susceptible animals, $HgCl_2$ induces autoimmunity [4,5]; in rats the kidney is often involved, with the precise pattern of glomerular injury varying according to the strain of rat involved [4,6,7]. The route of exposure seems relatively unimportant: susceptible rats develop autoimmune manifestations after inhalation of mercury vapour, the potency being similar to subcutaneous injections of mercuric chloride [8]. The characteristics of $HgCl_2$-induced autoimmunity indicate preferential activation of the Th2 subset of helper T cells, since there is polyclonal B cell activation and hyper-IgE [3]. Up-regulation of IL-4, a key cytokine produced by Th2 cells, has been shown in response to $HgCl_2$ in the mouse [3,9] and more recently in the rat, both *in vivo* [10] and *in vitro* [11]. The Th1/Th2 compartment is believed to exist in a state of dynamic equilibrium, with reciprocal interactions between these two cell types, the net result of an immune response depending upon the balance between Th1 and Th2 effects [12–14]. Treatment of susceptible rats with an antibody to the Th1-type subset before administration of $HgCl_2$ leads to exacerbation of aspects of the autoimmune syndrome [15], suggesting that the outcome depends upon the balance of Th1 *versus* Th2 cells.

Immunoregulation of $HgCl_2$-induced autoimmunity may also depend upon this balance: the autoimmune response spontaneously terminates even if $HgCl_2$ injections are continued [16], and there is evidence that previously defective Th1 functions are restored during this regulation phase [17]. Perhaps it is not surprising therefore that the immune stimulation induced by $HgCl_2$ is not solely confined to the Th2 compartment: there is also evidence that interferon-gamma (IFN-γ), usually regarded as a Th1 cytokine, is up-regulated after $HgCl_2$ *in vivo* in the rat, albeit to a lesser degree than IL-4 and with a different time course (Gillespie & Mathieson, in preparation). *In vitro*, $HgCl_2$ has complex effects on IFN-γ production: initially there seems to be enhanced IFN-γ production by spleen cells from both susceptible and resistant rat strains (Prigent, personal communication), but later IFN-γ production is suppressed in susceptible animals [18]. This effect seems to be mediated by nitric oxide (NO), since it could be abrogated by competitive inhibition of NO synthesis [19]. Furthermore, there is evidence that $HgCl_2$ leads to up-regulation of NO production in the spleen of susceptible rats [19]. Aged rats are less susceptible to $HgCl_2$-induced immunopathology, and such animals have an enhanced capacity for IFN-γ production, and thus a shift towards the Th1 compartment relative to younger animals. In summary, by directly up-regulating IL-4, and also by inducing the production of NO which inhibits IFN-γ, $HgCl_2$ leads to an autoimmune process in which there is tipping of the immunoregulatory balance towards the Th2 compartment. This challenges the dogma that Th1 cells provide the potentially auto-aggressive compartment and Th2 cells the regulatory role [13]: in some circumstances it seems that the opposite is true.

The cellular and molecular mechanisms by which $HgCl_2$ exerts its effects are not fully explained. The chemical has a propensity to bind to sulfhydryl groups of proteins and non-protein thiols, and other agents such as gold salts and penicillamine which induce similar autoimmune phenomena in susceptible animals [20,21] share this characteristic. Chemical modification of MHC class II molecules, T cell receptors, auto-antigenic peptides or some other cell-surface molecules has been postulated [3], but there is no direct evidence for these effects. $HgCl_2$ can induce apoptosis in T cells *in vitro* [22], and this effect may be enhanced by IL-4 [23]. Increasingly, molecular immunology has turned its attention to intracellular signalling events: an intriguing recent observation is that $HgCl_2$ induces aggregation of cell surface proteins such as CD4, CD3, CD45 and Thy-1 on T cells *in vitro*, followed by dramatic tyrosine phosphorylation of several cellular proteins, including the non-receptor protein tyrosine kinase $p56^{lck}$ [20]. These effects were dependent on the bivalent thiol reactivity of $HgCl_2$. However, it should be noted that very high concentrations of $HgCl_2$ were used, sufficient to induce rapid death of the cells [20]. Alteration of intracellular redox potential via effects on glutathione have been demonstrated *in vitro* after exposure of T cells to $HgCl_2$ (Aten, data presented at 9th International Congress of Immunology, San Francisco, July 1995); the effects of $HgCl_2$ on IFN-γ production by spleen cells *in vitro* differ between susceptible and resistant strains, and this seems to be at least partly explained by variations in sensitivity to the effects of depletion of reduced glutathione [18]. These observations do

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not readily explain a preferential effect on the Th2 cellular compartment. Mast cells from susceptible rat strains show sensitization by $HgCl_2$ *in vitro* [24], and also show alterations in intracellular redox potential after incubation with $HgCl_2$ (Oliveira, in preparation). Since mast cells produce IL-4 and an initial source of IL-4 is required for the generation of a Th2 response, one possibility is that the mast cell is a key cell in susceptibility to the induction of autoimmunity by $HgCl_2$.

In this issue (page 297), Coers and colleagues illustrate another mechanism whereby $HgCl_2$ may induce tissue injury [25]. They postulate that increases in circulating levels of IFN-γ and IL-4 may contribute directly to the induction of proteinuria by $HgCl_2$. In support of this hypothesis, they show impressive effects of IL-4 and IFN-γ on glomerular epithelial cells *in vitro*. They also report increased IFN-γ immunoreactivity in the kidneys of $HgCl_2$-treated rats; unfortunately, no data on IL-4 *in vivo* are provided, mainly because antibodies to rat IL-4 are not yet widely available. Similarly, the specificity of the *in vitro* effects attributed to IL-4 cannot be proved without specific neutralizing anti-IL-4 antibodies. The apparent potency of IL-4 in inducing alterations in viability of glomerular epithelial cells, monolayer integrity and matrix synthesis are somewhat surprising, and the relevance of these observations to the *in vivo* situation must await measurements of circulating IL-4 levels to see whether the concentrations used *in vitro* were physiological.

Nevertheless, these results illustrate another effector mechanism by which $HgCl_2$ may act. In animals predisposed to the development of a Th2 type response, $HgCl_2$ results in up-regulation of IL-4, leading to B cell activation and class-switching to IgE, associated in some cases with widespread tissue inflammation [26]. There may also be up-regulation of IFN-γ during the effector phase of the response. The data from Coers *et al.* show that the cytokines themselves may have previously unsuspected effects at the tissue level.

In terms of relevance to human disease, preferential Th2 activation may underlie asthma, allergic disease and possibly some forms of autoimmunity. Improved understanding of the mechanisms and consequences of Th2 activation can be expected to provide information which as well as being of basic immunological importance has possible clinical implications. The capacity of $HgCl_2$ to preferentially activate Th2 cells provides a powerful tool for the study of this cellular compartment.

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